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Measuring Levels of IL4, TNF Alpha and Cortisol among Diabteic Type 2 Individual in Samarra City

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Annotation: The current study involved sample collection from February 10, 2023, to June 5, 2023. Fifty samples, including 30 diabetic patients and 20 healthy controls, were collected from Samarra General Hospital and its clinics. Serum levels of tumor necrosis factor alpha (TNF-α), interleukin-4 (IL-4), and ketones were measured using a radioactive enzyme assay technique. The study results showed a significant increase in tumor necrosis factor alpha (TNF-α) levels by 273.0 ± 24.04 pg/ml in the patient group compared to the control group (58.5 ± 13.18 pg/ml), in addition to higher cortisol levels in the patient group (0.07 ± 3.94 pg/ml) compared to the control group (0.05 ± 1.53), while the results showed increase in interleukin-4 levels in the patient group (0.37 ± 0.158) compared to the control group (0.58 ± 0.107). We conclude from these results that elevated levels of inflammatory factors play an important role in detecting the development of type 2 diabetes in patients.

Keywords: 1- Diabetic type 2; 2- TNF alph; 3- cortisol; 4 IL4.

1. Introduction

Impaired insulin secretion is a fundamental aspect of type 2 diabetes. Insulin production varies greatly depending on insulin sensitivity to maintain normal blood glucose levels. The readiness index is used to assess the curvilinear relationship between insulin sensitivity and insulin secretion. Patients with type 2 diabetes typically exhibit a low readiness index, limiting their ability to increase insulin production in response to insulin resistance. Although absolute insulin levels in obese, insulin-resistant individuals may be higher than those in lean, insulin-sensitive individuals, these levels remain inadequate for the severity of their resistance [1].

Furthermore, the first-phase insulin response to glucose stimulation is impaired or completely absent in patients with type 2 diabetes. An elevated proinsulin-to-insulin (or C-peptide) ratio is also common in these individuals. Furthermore, peak insulin production and the potentiation of insulin response to non-glucose stimuli are significantly reduced, contributing to progressive hyperglycemia that becomes difficult to control over time. A persistent decline in beta cell function is another hallmark of the development of type 2 diabetes [2,3].

Hypercortisolism—defined as chronically elevated cortisol levels—can induce treatment-resistant hyperglycemia, which may be beyond the efficacy of modern pharmacological treatments for type 2 diabetes, including GLP-1 and GIP receptor agonists. Cortisol is also associated with increased inflammation in type 2 diabetes patients with microvascular complications such as retinopathy, neuropathy, and nephropathy, suggesting its role in inflammatory pathways that contribute to disease progression [4].

There is strong evidence that dysfunctions of the hypothalamic-pituitary-adrenal (HPA) axis mediate the relationship between psychosocial stress and metabolic dysfunction. Cortisol, a glucocorticoid hormone and a key component of the HPA axis, exerts anti-insulin effects by stimulating hepatic gluconeogenesis and inhibiting peripheral glucose uptake. Activation of the HPA axis under physiological or psychological stress leads to the release of corticotropin-releasing hormone (CRH) from the hypothalamus. CRH stimulates the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH), which in turn stimulates the adrenal cortex to secrete cortisol. A negative feedback loop involving cortisol leads to decreased secretion of both ACTH and CRH [5].

Additionally, activation of the HPA axis is accompanied by stimulation of the sympathetic nervous system, leading to the release of catecholamines and interleukin-6 (IL-6), which initiates a cascade of pro-inflammatory cytokines. Chronic stress can impair the feedback mechanisms that regulate these systems, leading to persistently elevated levels of cortisol, catecholamines, and inflammatory markers [6].

Regarding inflammation, tumor necrosis factor alpha (TNF-α) and interleukin-4 (IL-4) are well-characterized pro-inflammatory cytokines that contribute to insulin resistance. Several studies have also indicated a pathogenic role for IL-6 in this context [7]. Tumor necrosis factor alpha (TNF-α) is an adipokine secreted primarily by macrophages and, to a lesser extent, adipocytes and other cells. It plays a crucial role in the inflammatory response in the acute phase. Dysregulation of TNF-α metabolism has been shown to be associated with the onset and progression of type 2 diabetes [8,9].

2-Materials and Methods

Collect of sample

Blood samples were collected from patients who had diagnosed with DM2 by withdrawing 5 ml of venous blood using a medical syringe with a capacity of (5 ml), then placed in tubes containing gel to separate the blood, then separated at a speed of 3000 rpm for 5 minutes for the purpose of separating the serum. The blood was separated and the serum was taken, and the sera were distributed in Eppendorf tubes with a capacity of 1.5 ml. Then the samples were stored at a

temperature of -20 C until ELISA tests was performed. The samples were divided into two groups: The first group: the control group, which consists of 20 samples. The second group: the patient group, which consists of 30 samples.

Laboratory diagnosis

Enzyme-linked immunosorbent assay

ELISA kit is used to measure IL-4 ,TNF and cortisol levels in serum quantitatively. The kit was provided by Bioassay Technology Laboratory (Nanhu Dist, Jiaxing, Zhejiang, China).

Statistical Analysis : using Statistical analysis was performed using Chi-square andUse the Duncan multi-range test below a likelihood level ($P<0.05$).

3- Results and dissection

- Levels of IL4 in study groups

Table (1): Shows the average levels of interleukin -4 in the patient group compared to the control group (pd/ml).

Interleukin -*4*	IL-4 Mean± S.D
Patient group	0.37 ± 0.158
Control group	0.58 ± 0.107

Type 2 diabetes mellitus (T2DM) is increasingly recognized as an immune-mediated chronic inflammatory condition, in which leukocyte infiltration and elevated cytokine levels contribute to alveolar bone loss, synovitis, and joint damage. Interleukin-4 (IL-4), a cytokine primarily produced by T-helper 2 (Th2) cells, plays a dual role in adaptive immunity by promoting B-cell proliferation and exerting potent anti-inflammatory effects. IL-4 inhibits the secretion of pro-inflammatory cytokines—most notably TNF-α, IL-1β, IL-6, and IL-8—from monocytes and macrophages [10,11].

This regulatory mechanism helps prevent excessive inflammation and reduce tissue damage. At the molecular level, IL-4 signals via the JAK-STAT6 pathway, polarizing macrophages toward an anti-inflammatory M2 phenotype, which is also characterized by increased production of IL-10 and TGF-β—cytokines essential for resolving inflammation and supporting tissue repair [12].

In the context of type 2 diabetes, patients often exhibit dysregulated inflammatory cascades with significantly lower IL-4 expression in periodontal tissue compared to non-diabetics[14], which may partly explain their increased susceptibility to inflammatory complications such as periodontitis[15].

Studies have shown that decreased IL-4 in diabetic patients is associated with increased bone resorption and impaired wound healing, underscoring a compromised immune response [16]. Together, these findings suggest that IL-4 plays a pivotal role in immune modulation by suppressing pro-inflammatory cytokines, directing macrophages toward a reparative phenotype, and possibly mitigating bone and joint damage in inflammatory diseases. Therefore, IL-4 represents a promising target for therapeutic intervention in inflammatory bone diseases associated with type 2 diabetes.

- **Levels of cortisol in study groups**

Table (2): Shows the average cortisol values in the patient group and their comparison with the control group in nanomoles/liter.

cortisol	**Cortisol Mean ±S.D**
Patient group	3.94 ±0.07
Control group	1.53±0.05

Several studies have shown that patients with type 2 diabetes often exhibit elevated cortisol levels, which is commonly known as the stress hormone. This elevation is believed to be linked to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, contributing to increased insulin resistance and poor glycemic control [17] . High cortisol levels can impair carbohydrate metabolism and increase the risk of cardiometabolic complications [18] . Additionally, chronic cortisol elevation promotes a pro-inflammatory state, worsening metabolic disturbances in diabetic individuals [19] . Therefore, monitoring cortisol levels may provide valuable insights into disease progression and management strategies.

- **The level of TNF-alpha in study groups-**

Table (3): Shows the average TNF-alpha values in the patient group and their comparison with the control group in pg/ml.

Tumor necrosis factor alpha	TNF-α Mean ± SD
Patients	273.0 ± 24.04 pg/ml
Controls	58.5 ± 13.18 pg/ml

TNF-α is a key pro-inflammatory cytokine involved in immune responses, including fever induction, which is a defense mechanism against pathogens. It acts synergistically with other inflammatory mediators such as interferon-alpha (IFN-α) and natural killer (NK) cells to combat type 2 diabetes (DM2)-associated inflammation[20]. TNF-α directly influences the brain and peripheral tissues by stimulating the production of additional cytokines like interleukin-10 (IL-10), IFN-α, IL-8, and other inflammatory molecules[21]. This cytokine network coordinates to activate NK cells, which are critical components of the innate immune system and provide early defense against microbial infections and abnormal cells[22]. The persistent activation of TNF-α and related cytokines contributes to chronic inflammation observed in DM2, influencing disease progression and complications [23]. Conclusion

Conclusion

The results of this study highlight a significant association between elevated inflammatory markers and type 2 diabetes (T2DM). Diabetic patients showed significantly higher serum levels of tumor necrosis factor alpha (TNF-α) compared to healthy controls, indicating a pronounced inflammatory response in T2DM patients. Additionally, cortisol levels were elevated in the diabetic group, reinforcing the role of stress-related hormonal imbalance in the development of diabetes. In contrast, interleukin-4 (IL-4) levels were higher in diabetic patients, suggesting a potential immune-modulatory imbalance. These findings underscore the critical role of chronic inflammation and immune dysfunction in the pathogenesis of type 2 diabetes, underscoring the need for further research into anti-inflammatory therapeutic strategies for diabetes management.

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